



## Methoxsalen loaded chitosan coated microemulsion for effective treatment of psoriasis

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### Abstract

Methoxsalen has been used for the treatment of psoriasis. In order to develop alternative formulations for the topical administration of methoxsalen, chitosan coated microemulsion were evaluated as delivery vehicle. Microemulsions were prepared using water, soyabean oil. Egg phosphatidylcholine, ethanol and coated with chitosan. They were characterized for shape and surface morphology, droplet size and size distribution, zeta potential, pH and viscosity. The ability of the system to deliver into the skin was evaluated using dialysis membrane and human cadaver skin. The in vitro permeation data showed that the novel system cumulative amount released was 18.75 % lesser than the microemulsion. These studies clearly show that methoxsalen loaded chitosan-coated microemulsion provides control release of methoxsalen with retention on the skin. Therefore may be appropriate vehicle for topical delivery of methoxsalen.

**Keywords:** Microemulsions; Soyabean; Methoxsalen; Chitosan

### Introduction

Microemulsions and other related colloidal system have received increased attention during the past few years. Formulations based on microemulsions have several interesting characteristics viz, enhanced drug solubilization, good stability, and ease of manufacturing [1]. Although microemulsion can be used to deliver drugs via several routes; the system has been extensively studied as vehicle for topical administration [2]. Chitosan coated microemulsion are the novel carrier system. Generally emulsions of micron size are coated with chitosan. It is clear, milky thermodynamically stable mixture of oil, surfactant, and co-surfactant. This emulsion offers a significant advantage of increasing solubility of active agents for delivering active agent through skin. Advantages of this system are high biodegradability and low toxicity,

reduction in the clearance rate, increase in contact time with skin, ease of manufacturing etc [3].

In case of psoriasis, methoxsalen has been administered topically or systemically. Earliest topical formulation such as ointment, cream, and lotion produced a persistent hyperpigmentation [4]. Then it was replaced by oral therapy. But bioavailability of methoxsalen is highly variable because of its low water solubility and marked first pass effect. It also causes nausea, nervousness and mental depression after oral administration, [5] because of these short coming topical deliveries of methoxsalen by means of some topical formulation was attempted.

The aim of the present study was to develop and evaluate a novel delivery system as chitosan coated emulsion for the topical administration of Methoxsalen.

The benefits are: Prolonged effect, avoid first pass metabolism, reduction in adverse effect, retention on the skin, large contact area of drug as microemulsion.

## Materials and methods

### Materials

Methoxsalen was obtained from Inga Laboratories Pvt. Ltd, Mumbai, as a gift sample. Isopropyl myristate (IPM), oleic acid (OA), ethyl oleate (EO) and Soyabean oil were purchased from CDH Laboratory Pvt. Ltd Mumbai, Egg phosphatidylcholine, ethanol were purchased from SD Fine Chemical, Mumbai, Chitosan and dialysis membrane was purchased from HiMedia Laboratories Pvt. Ltd. Mumbai. Excised human cadaver skin from the abdomen was obtained from Chhattisgarh Institute of Medical Sciences, Bilaspur, India. Other chemicals used were of analytical grade.

### Method of preparation

#### Construction of pseudo-ternary phase diagram

In order to find out the range of existence microemulsion, pseudo-ternary phase diagrams were constructed using the water titration method at ambient temperature 25°C. Three phase diagrams were prepared with the 1:1, 2:1 and 3:1 weight ratios respectively of egg phosphatidylcholine to ethanol. Here egg phosphatidylcholine and ethanol act as surfactant and cosolvent respectively. For each phase diagram at specific surfactant/cosolvent weight ratio, the ratios of soyabean oil to the mixture of surfactant and cosolvent were varied as 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1. The mixtures of oil, surfactant and cosolvent at above weight ratios were diluted with water dropwise, under moderate magnetic stirring. After being equilibrated, the mixtures were assessed visually and determined as being microemulsion, crude emulsions or gels [6].

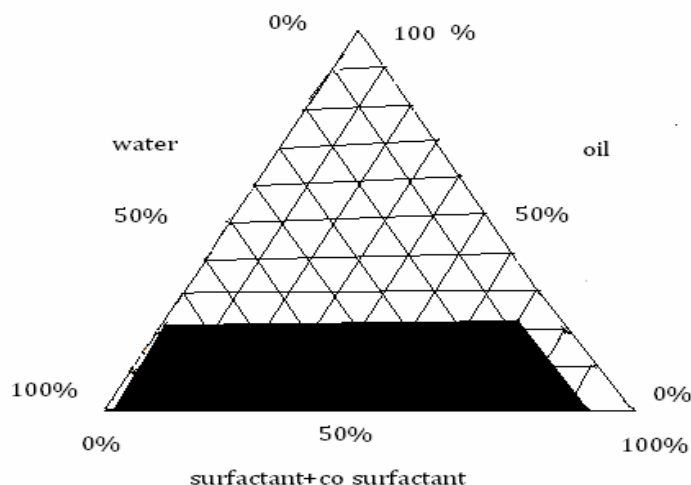
#### Preparation of microemulsion

A series of microemulsion formulations were prepared using soyabean oil as the oil phase, egg phosphatidylcholine as a surfactant and ethanol as cosolvent. In all the formulations, the amount of methoxsalen was kept constant. Accurately weighed methoxsalen was placed in a beaker; oil, surfactant, and cosolvent were added. Then the components were mixed by gently stirring with magnetic stirrer and

water was added drop-wise. The mixture was stored at room temperature until further use [3].

#### Preparation of chitosan coated microemulsion

Initially 2% aqueous solution of acetic acid was prepared, from which 0.25 ml of this solution was mixed with 2 mg of chitosan to obtain a homogenous mixture. Then the prepared microemulsion was added to equal amount of the chitosan mixture. The concentration of chitosan in the microemulsion was 4mg/ml [3].



**Figure1. Phase diagram showing surfactant co-solvent in ratio (3:1)**

### Characterization

#### Surface morphology and shape

Microstructure of the formulation was characterized by TEM. The formulation (5 micro liters) was diluted with water (1 ml) in a volumetric flask and sonicated for 5 minutes. Then one drop of the solution was placed on a carbon coated copper grid. One drop of 1% phosphotungstic acid was added to it. The superfluous phosphotungstic acid on sample was wiped off by filter paper and air dried for a minute. Finally the shape and size of formulations were examined by transmission electron microscope [7].

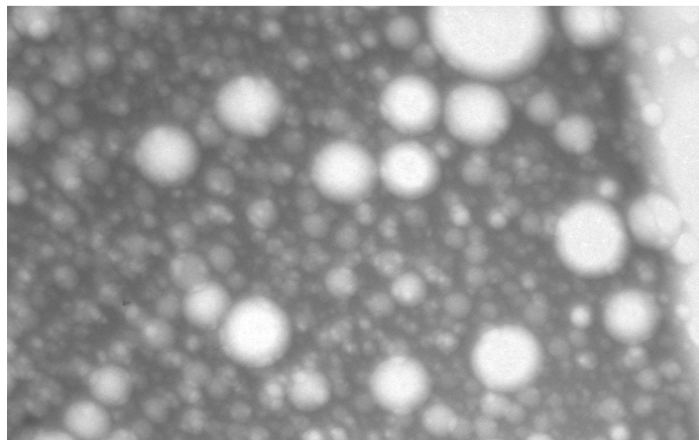
#### Droplet size and Size distribution

Droplet sizes of microemulsion and chitosan-coated microemulsion were determined by using dynamic light scattering method using melvern zeta master (MAL, 500962, Malvern, UK). One ml of formulation was diluted to 10 ml in a test tube and gently mixed

using a glass rod. The resultant emulsion was then subjected for particle size analysis [8].

### Zeta potential

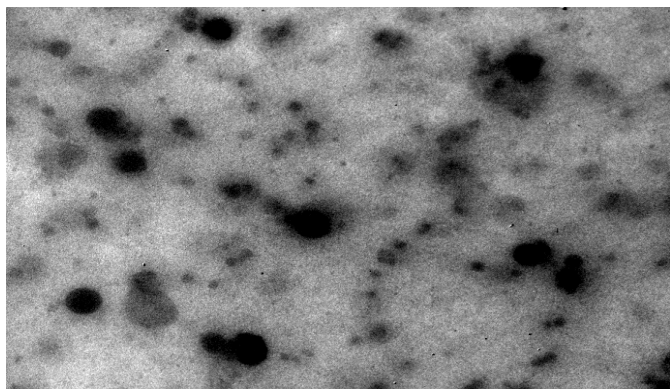
Zeta potential of formulations was determined by using dynamic light scattering method. (MAL, 500962, Malvern, UK). One ml of formulation was diluted to 10 ml in a test tube and gently mixed using a glass rod. The resultant emulsion was subjected for zeta potential analysis [7].



**Figure 2. Transmission electron microscopy image of formulation E3**

### Measurement of pH

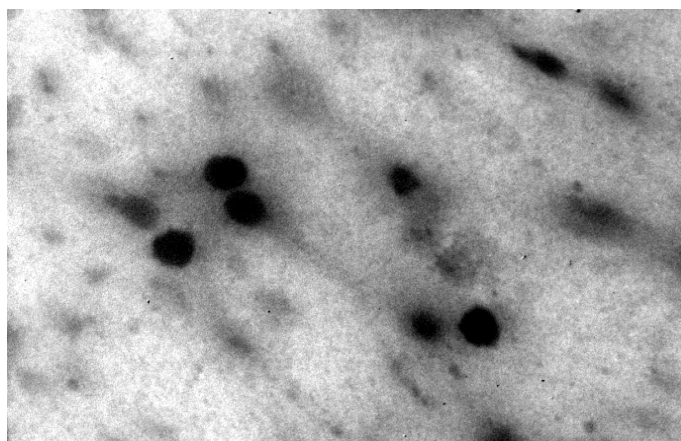
The pH values of the chitosan-coated microemulsion were measured at 25<sup>0</sup> C using digital pH-meter (Systronic digital pH meter 335, India). About 2.5 gm of the emulsion was dispersed in 25 ml of distilled water .the pH meter was first calibrated with a known pH solution. Then the pH of prepared formulations was determined [7].



**Figure 3. Transmission electron microscopy image of formulation E3C**

### Measurement of viscosity

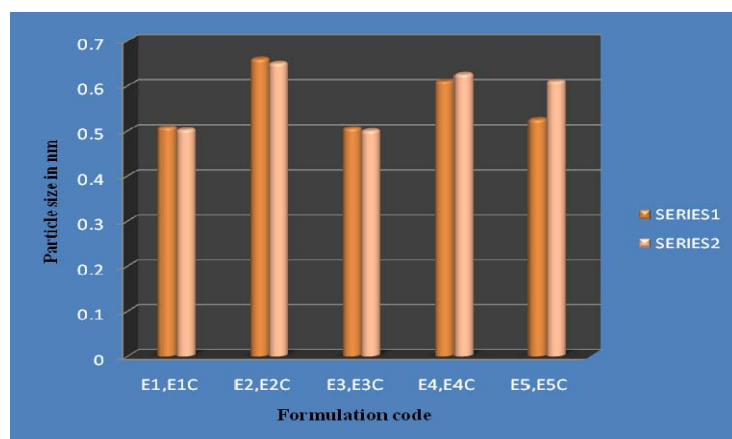
The viscosities of the chitosan-emulsion were measured at 25<sup>0</sup>C using digital viscometer (Model DU-II, Brookfield, USA) [7].



**Figure 4. Transmission electron microscopy image of formulation E2C**

### In vitro permeation studies

Selection of receptor solution used for permeation experiments is critical in case of topical application because they mimic the in vitro situation. Buffer solutions are the most commonly used media for lipophilic compounds. Dialysis membrane (average flat width- 29.31 mm, average diameter- 17.5mm, capacity factor- 2.41ml/cm) was used as an artificial membrane for preliminary in vitro studies because of simplicity, homogeneity and uniformity. This membrane was hydrated in PBS (pH 5.5) for 24 hours prior to use.



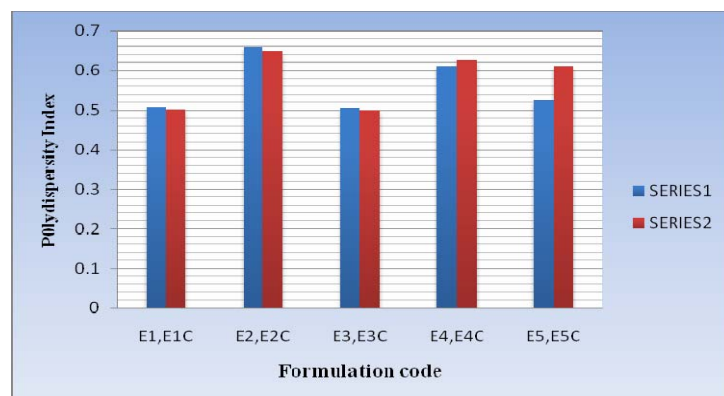
**Figure 5. Graphical representation of particle size**

The permeation studies were carried out using a modified Franz Diffusion cell. The effective permeation area of the diffusion cell and receptor cell volume was 2.545 cm<sup>2</sup> and 15ml respectively. The temperature of receptor fluid was maintained at 37±1°C.

**Table 1. Composition of different microemulsion formulation**

Component	E1	E2	E3	E4	E5
Methoxsalen (mg)	0.05	0.05	0.05	0.05	0.05
Soyabean oil (%)	4	6	8	10	11
Egg phosphatidylcholine (%)	15	30	36	24	39
Ethanol (%)	5	10	12	8	13
Water (%)	72	53	44	52	27

The receptor compartment contained PBS (pH5.5). Dialysis membrane was mounted between the donor and receptor compartment. 1 gm formulation of drug was applied to the upper side of membrane in donor compartment. Samples were withdrawn through the sampling port of the diffusion cell at predetermined intervals over 24 hours and analyzed spectrophotometrically [9].



**Figure 6. Graphical representation of Polydispersity index**

An equal volume of fresh PBS (pH 5.5) maintained at 37±1°C was replaced into the receptor compartment after each sampling. Cartesian plots of cumulative amount of drug in receptor compartment versus time

were plotted. Flux (Jss, µg/cm<sup>2</sup>/hr.) was calculated from the slope of the steady state portion of these graphs.

**Table 2. Particle size distribution and Polydispersity index**

Formulation code	Particle size in nm	Polydispersity Index
E1	512	0.507
E2	510	0.659
E3	391	0.505
E4	372	0.610
E5	425	0.525
E1C	512	0.502
E2C	519	0.650
E3C	395	0.500
E4C	365	0.625
E5C	420	0.610

### **Ex vivo permeation studies through human cadaver skin**

The skin which was collected not more than 3 days after postmortem was stored at 4°C in formalin. The skin was immersed in purified water at 60°C for 2 minutes. Then it treated to remove hair and subdermal tissues [10].

**Table 3. Zeta potential of the formulation**

Formulation code	Zeta potential (mV)
E1	-37.8
E2	-40.2
E3	-49.1
E1C	19.4
E2 C	20.4
E3C	23.8

The penetration studies were carried out by using Franz- type diffusion cells. The cell had a diffusion surface area of 2.545 cm<sup>2</sup> and a volume of 15ml in the receptor compartment.

**Table 4. pH value and viscosity of chitosan-coated microemulsion**

Formulation code	pH	Viscosity (Pa.S)
E1C	5.10	20.2
E2C	5.68	22.1
E3C	5.48	23.4
E4C	5.75	21.3
E5C	5.60	23.8



**Table 5. Data of cumulative amount release from chitosan-coated microemulsion and one microemulsion through dialysis membrane**

Sample collection time (hr)	Cumulative amount released( $\mu\text{g}$ )					
	E1C	E2C	E3C	E4C	E5C	E3
1	4.00	3.12	5.89	5.64	4.27	15.35
2	13.5	7.25	11.50	10.65	9.24	19.43
3	20.8	17.02	23.53	20.53	13.73	24.42
4	27.20	26.50	28.90	25.64	24.17	34.43
5	55.50	38.30	70.82	37.86	40.30	62.82
6	157.62	123.96	160.64	128.29	93.83	184.96
12	170.30	163.60	210.85	189.43	134.42	230.58
18	263.32	275.92	284.81	272.64	220.34	332.45
24	281.52	287.67	292.32	283.94	253.76	372.59

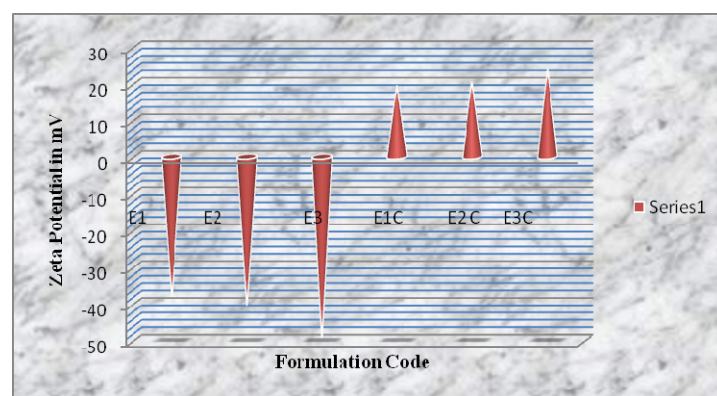
Human skin was mounted horizontally with the stratum corneum side up; dividing the cell into two compartments i.e. the donor and the receptor compartments.

**Table 6. Data of % of drug release and flux through dialysis membrane**

Formulation code	% Release in 24 hr	Flux ( $\mu\text{g}/\text{cm}^2/\text{hr}$ )
E1C	51.18	5.42
E2C	52.38	5.63
E3C	53.09	6.45
E4C	50.9	5.28
E5C	50.8	5.17
E3	67.63	8.12

The dermal side of skin was immersed in PBS (pH 5.5) and the formulation was in contact with outer skin layer. The receptor was filled with pH 5.5 saline phosphate buffered solution. The receptor compartment was maintained at  $37 \pm 0.5^\circ\text{C}$  using water bath with thermostatic plate of magnetic stirrer and stirred at 300 rpm by Teflon coated magnetic bead fitted to a magnetic stirrer. Formulation (1gm) was applied in the donor compartment. Pseudo- sink conditions were maintained throughout the duration of the permeation experiments. The duration of experiments was 24 hour. At prefixed intervals 1 ml of the receptor phase was withdrawn and analyzed spectrophotometrically to determine the amount of permeated methoxsalen. The withdrawn volume was replaced with fresh medium and

a correction for dilution was introduced. The chamber was kept protected from light. Cartesian plots of cumulative amount of drug present in receptor compartment versus time were plotted. Flux ( $J_{ss}$ ,  $\text{mg}/\text{cm}^2/\text{hr}$ ) was calculated from the slope of the steady state portion of these graphs [7].



**Figure 7. Graphical representation of zeta potential**

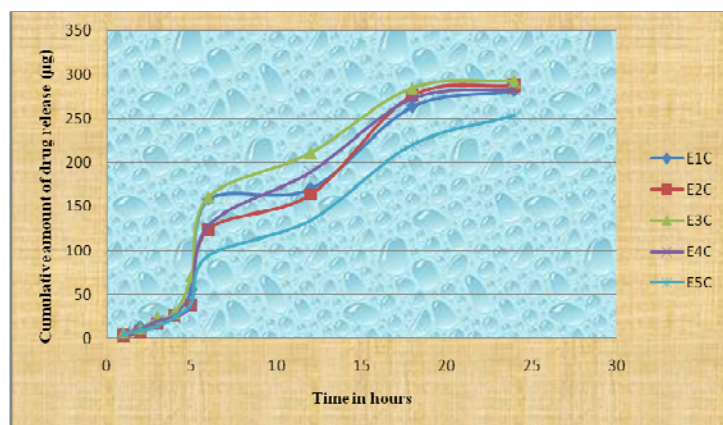
### Skin deposition studies

At the end of the permeation studies, the skin surface was washed five times with ethanol: PBS (pH 5.5) 1:1 solution, then with water to remove excess drug from the surface. The skin was then cut into small pieces. The tissue was further homogenized with ethanol: buffer solution (pH 5.5) 1:1 and left for 6 hr. at room temperature. After shaking for 5 min and centrifugation for 5 min at 5000rpm, the drug concentration at supernatant was determined spectrophotometrically [1].

### Stability study

Stress conditions normally applied for evaluating the stability of microemulsion includes aging, temperature

and centrifugation. The main parameters which are used to determine the stability is phase separation, viscosity and globule size distribution [11]. Selected formulations were kept at three different temperatures i.e. 4°C, 25°C, 45°C and observed for phase separation and particle size for about 45 days. Formulations were also subjected to centrifugation at 3000, 5000, 10,000 rpm for 15 minutes at room temperature and samples were withdrawn after a particular time interval and observed for phase separation and average particle size.



**Figure 8. Comparison of cumulative amount release from chitosan-coated microemulsion through dialysis membrane**

## Results

### Pseudoternary phase diagram

The phase diagrams are used to find out the microemulsion region. The studied systems were composed of soyabean oil, egg phosphatidylcholine, ethanol and water. The pseudoternary phase diagrams with various weight ratios of egg phosphatidylcholine and ethanol were prepared and the best result i.e. (3:1 ratio) is shown the figure 1. The shaded regions in these figures represents the microemulsion formation regions. The microemulsion region was changed slightly in size with increasing ratio of egg phosphatidylcholine to ethanol.

### Preparation of microemulsion and chitosan coated-microemulsion

The compositions of selected formulations are shown in the table1, microemulsion were produced using these compositions and then the corresponding chitosan-coated emulsion (E1C, E2C, E3C, E4C, and E5C) was

prepared by mixing equal amount of aqueous chitosan solution (4mg/ ml) to the above microemulsion.

### Surface morphology and shape

The surface characteristics of microemulsion and chitosan-coated microemulsion were performed on three formulations selected on the basis of particle size viz E3, E3C, E2C formulations using transmission electron microscopy (TEM; CM12 Philips, USA). The TEM micrographs of these formulations are presented in Fig No 2, 3, and 4.

**Table 7. Data of cumulative amount release from chitosan-coated microemulsion and one microemulsion through human cadaver skin**

Sample collection time (hr)	Cumulative amount released (µg)	
	E3	E3C
0.5	16.32	5.62
1	20.40	16.32
1.5	25.50	24.30
2	35.85	30.34
3	73.82	42.57
4	108.58	76.90
5	235.25	152.61
6	245.30	170.78
8	290.32	220.97
12	360.42	300.81
24	412.35	308.42

### Droplet size and Size distribution

The selected formulations were subjected for analysis by Malvern Zeta master (MAL, 500962, Malvern, UK). The results are shown in table 2 and graphical representation in fig no. 5 and 6.

### Zeta potential

Zeta potential of the selected formulations was determined by Malvern Zeta master (MAL, 500962, Malvern, UK). The results are shown in table no. 3 and graphically represented in fig no.7.

### Measurement of pH

The pH values of the chitosan-coated microemulsion were measured at 25°C using digital pH-meter (Systronic digital pH meter 335, India.). The results obtained are given in the table no.4.

### Measurement of viscosity

Viscosity of the formulation plays an important role because it affects the permeation rate and stability. Viscosities of selected formulations were measured by viscometer (Model DU-II, Brookfield, USA) and the results are given in the table no. 4.

**Table 8. Data of % Release in 24 hr and Flux from excised human skin**

Formulation code	% Release in 24 hr	Flux ( $\mu\text{g}/\text{cm}^2/\text{hr}$ )
E3	74.87	6.25
E3C	56.12	5.43

### In vitro permeation studies

Drug permeation study was conducted through cellophane membrane with selected optimized formulations (E1C, E2C, E3C, E4C, and E5C) and one microemulsion (E3) was studied by using locally fabricated Franz diffusion cell. The cumulative amount of methoxsalen released from chitosan-coated microemulsion formulations through an artificial membrane at various sampling time are presented in table no.5 and figure no. 8. The comparison between one microemulsion and one chitosan-coated microemulsion is also given in figure no. 9. The % of drug release and fluxes are also given in the table no. 6.

**Table 9. Data of % of drug deposited after 24 hour**

Formulation code	% of drug deposited after 24 hour
E3	24.13
E3C	41.88

### Ex vivo permeation studies through human cadaver skin

To actually mimic the *in vivo* skin permeation study the permeation studies were performed using excised human cadaver skin mounted on Franz diffusion cell. The optimized formulations E3 and E3C were utilized to predict release profile from chitosan-coated microemulsion through human cadaver skin. The results are shown in the table no.7, and graphical

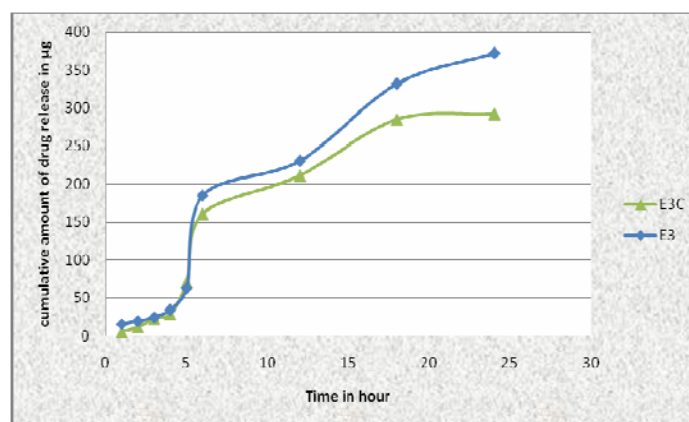
representations are shown in the fig no.10. The % of drug release and the flux are given in the table no. 8.

### Skin deposition studies

The amount of drug deposited after 24 hour of the permeation study through human cadaver skin was determined and presented in the table no. 9.

### Stability study

Selected formulations were kept at three different temperatures i.e.  $4^{\circ}\text{C}$ ,  $25^{\circ}\text{C}$ ,  $45^{\circ}\text{C}$ . The formulations E1C, E2C, E4C and E5C shows phase separation at 45 days. But the formulation E3C was found stable at all temperatures and for 45 days. The particle size of the formulation (E1C, E2C, E4C and E5C) also change at higher temperature. The formulation E1C, E2C, E3C shows no change in phase separation or particle size distribution after centrifugation and the formulation E4C, E5C show change in their particle size.



**Figure 9. Comparison of cumulative amount release from chitosan-coated microemulsion and microemulsion through dialysis membrane**

### Discussion

From the phase diagrams it was concluded that the area of microemulsion formed was greater in the surfactant, co-solvent ratio 3:1. So this ratio can be chosen for microemulsion preparation.

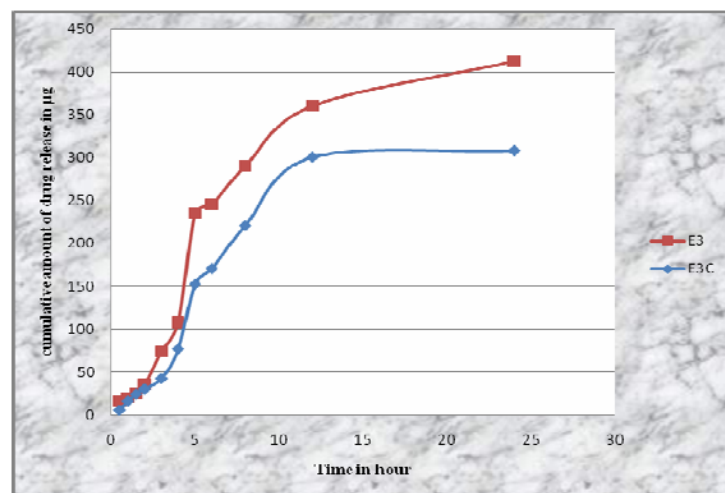
TEM imaging of chitosan-coated microemulsion revealed that droplets were spherical in shape even after addition of chitosan. The result obtained from the DLS study (particle size) was also confirmed spherical shape of particles.

The particle size of microemulsion and chitosan-coated microemulsion in table no. 2. Particle size of

formulation E3 was smallest among other formulations. Polydispersity Index of this formulation was also lesser than other formulations. The particle size of chitosan-coated microemulsion was also relatively constant as compared to their corresponding microemulsion. From this it is concluded that particle size does not vary considerably with coating by chitosan. (Nagamoto et al.2004) The Polydispersity Index of all the formulations is also comparable and does not vary significantly. This concludes that all the formulations possess uniform particle size droplets.

Zeta potential of all the prepared microemulsions are greater than -30 mv so the formulations can be considered. Further the Zeta potential of chitosan-coated microemulsion is positive. From this it is concluded that the emulsion is coated by chitosan.

The pH value of the formulation E3C is 5.48 which is near the value of the skin, so it not produce any adverse effect or irritation on the skin. The viscosities of all formulations are nearly same and suitable for topical delivery.



**Figure 10. Graphical presentation of cumulative drug release across excised human skin**

In vitro release with dialysis membrane revealed that all formulations exhibit good in vitro release characteristics. The formulation E3C shows maximum flux. From the fig no 8 it is inferred that microemulsion E3C has higher permeation than the chitosan-coated microemulsion. Percentage of drug release is also higher for the formulation E3C. From the fig 9 it is concluded that chitosan-coated microemulsions are having control release than other microemulsions. The

cumulative amount of drug release and the flux was found in the range of 50.8-53.09 % and 5.28-56.45  $\mu\text{g}/\text{cm}^2/\text{hr}$  respectively. The cumulative amount of drug release was compared with the plain microemulsion and found that chitosan-coated microemulsion showed 18.75% lesser release. Either no lag or small lag phase was observed with formulations. This suggests a quick onset. The permeation of methoxsalen through human cadaver skin was determined which mimic most in vivo condition for permeation of the drug. The amount of drug received in receptor compartment during 24 hr study period from formulations (100 mg) was determined with sampling at periodic intervals. From result represented graphically (fig no.10) it was seen that the formulations possess control release through skin. The transdermal flux calculated is low, exhibit that there is no or very small lag time to reach the receptor solution. The % release of methoxsalen through human cadaver skin in 24 hours from chitosan-coated microemulsion is lower than the general microemulsion. The amount of drug deposited after 24 hour is higher than the microemulsion. This result indicates it can be used for effective topical delivery.

Stability studies were conducted for chitosan-coated microemulsion by storing the E3C formulation at refrigeration temperature (4°C) room temperature (25°C) and accelerated temperature (45°C) for 45 days. From the result it is concluded that formulation is stable at low and room temperature. The formulations were subjected for centrifugation study at 3000, 5000 and 10000 rpm. The formulations showed no change in phase separation or particle size distribution. This suggested that formulations were stable and can be stored for longer period of time.

These studies clearly show that methoxsalen loaded chitosan-coated microemulsion provide control release with retention on the skin.

Additional advantage of chitosan-coated microemulsion is ease of application on skin. The formulation is easy to scale up as the procedure is simple and does not involve lengthy procedure and unnecessary use of pharmaceutically unacceptable additives.

In conclusion, we can state that aim of project has been met viz., to circumvent the drawbacks of convention dosage form and retention on the skin. Thus chitosan-coated microemulsion acts as a novel system for topical delivery of methoxsalen.



## Conclusions

These studies clearly show that methoxsalen loaded chitosan-coated micro emulsion provide control release with retention on the skin. Additional advantage of chitosan-coated micro emulsion is ease of application on skin. The formulation is easy to scale up as the procedure is simple and does not involve lengthy procedure and unnecessary use of pharmaceutically unacceptable additives. In conclusion, we can state that aim of project has been met viz., to circumvent the drawbacks of convention dosage form and retention on the skin. Thus chitosan-coated micro emulsion acts as a novel system for topical delivery of methoxsalen.

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## References

1. Baroli B, Lopez Quintele MA, Delgadocharr MB, Fadda AM, Blanco-Mendz J. microemulsion for topical delivery of 8-methoxsalen., *J.contol Rel.*, 2000; 69: 209-218.
2. Gasco M R. Microemulsions in the Pharmaceutical Field: Perspectives and Application, *Industrial Applications of Microemulsions*, Mercel Dekker Inc, New York., 1997: 97 – 122.
3. Nagamoto T, Yoshiyuki H Kozo T, Yoshie M. Novel Chitosan Particles and Chitosan-Coated Emulsions Inducing Immune Response via Intranasal Vaccine Delivery, *Pharmaceutical Research*, 2004; 10 : 671-674.
4. Wolff, K.Side effect of psoralen photochemotherapy, *Br.J.Dermatol.* 1990;122:43-52.
5. Martindale. The extra pharmacopoeia, 30 th edition, the pharmaceutical press, London, 1993.
6. Maghraby El, Gamal M. Transdermal delivery of hydrocortisone from eucalyptus oil micro emulsion, *Effects of co surfactants Int. Jour. Pharm.*, 2007; 355:285–292.
7. Pittermann W, Jackwerth B, Schmitt M. the isolated perfused BovineUdderSkin. A new in vitro model for the assessment of skin penetration and irritation. *Toxic. in Vitro*, 1997; 10: 17-21.
8. Valenta C, Biruss B, The advancement of polymer addition to an non ionic oil in water microemulsion for the dermal delivery of progesterone. *Int. jour. Pharm.*, 2008; 349:269-273.
9. Agrawal GP, Jain SK, Pancholi SS, Agrawal S. Provesicular transdermal drug delivery system for Ethinylestradiol and Levonorgestrel for contraception and hormonal replacement therapy *Indian J. Pharm. Sci.*, 2003, 65: 620-627.
10. Chen H, Mou D, Du D, Mao C, Wan J, Xu J Yang. Hydrogel-thickened nanoemulsion system for topical delivery of lipophilic drugs *Int. Jour. Pharma.*, 2008; 353: 270–276.
11. Lachman L, Lieberman H, Kanig J.The theory and practice of industrial pharmacy, Varghese publishing house, Mumbai, 1996; 3<sup>rd</sup> edition: 535-536.